OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

APPLICATION FOR IRB APPROVAL OF HUMAN SUBJECTS RESEARCH
version 16-Feb-2005

For IRB Use
Behav Bio Dent Nurs PH
IRB Study #_____
Rec'd_____

Part A-1. Contact Information, Agreements, and Signatures

Date of Application: August 3, 2005 Revised: August 28, 2007

Title of Study: Respiratory effects of short-term low-level chlorine gas exposure

Name of Principal Investigator: Howard R. Kehrl M.D. *(For IRB communication purposes, a trainee/student may be listed as PI. However, a faculty advisor must be identified, who holds ultimate responsibility for ensuring that this project complies with all University, regulatory, and fiscal requirements.)*
Department: U.S. Environmental Protection Agency Mailing address/CB #: 7315
UNC-CH PID: 704509872 Pager: 216-6011
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For trainee-led projects: ☐ undergraduate ☐ graduate ☐ postdoc ☐ resident ☐ other
Name of faculty advisor:
Department: Mailing address/CB #:
Phone #: Fax #: Email Address:

Name, phone number, email address of project manager or coordinator, if any:

List **all other project personnel** including co-investigators, and anyone else who has contact with subjects or identifiable data from subjects: Annie Jarabek, Martin Case, Andy Ghio MD, William Bennett PhD, Dave Peden MD, Milan Hazucha MD. PhD, Lynne Newlin-Clapp, Mary Ann Bassett RN, Debbie Levin RN, Tracey Montilla RN, Martha Almond RRT, Carol Robinette MS CPFT, Keegan Musgrove-Wesley MS, Margaret Herbst RN, Martha Sue Carraway MD, Bob Devlin PhD

Name of funding source or sponsor: U.S. Environmental Protection Agency
☐ not funded ☐ Federal ☐ State ☐ industry ☐ foundation ☐ UNC-CH
☐ other (specify):

Sponsor or award number:

Principal Investigator: I will personally conduct or supervise this research study. I will ensure that this study is performed in compliance with all applicable laws, regulations and University policies regarding human subjects research. I will obtain IRB approval before making any changes or additions to the project. I will notify the IRB of any other changes in the information provided in this application. I will provide progress reports to the IRB at least annually, or as requested. I will report promptly to the IRB all unanticipated problems or serious adverse events involving risk to human subjects. I will follow the IRB approved consent process for all subjects. I will ensure that all collaborators, students and employees assisting in this research study are informed about these obligations. All information given in this form is accurate and complete.

_____ _____

Signature of Principal Investigator Date

For faculty advisor, if the PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that this study complies with all the obligations listed above for the PI.

_____ _____

Signature of Faculty Advisor Date

Department or Division Chair, Center Director (or counterpart) of PI: (or Vice-Chair or Chair's designee if Chair is investigator or otherwise unable to review): I certify that this research is appropriate for this Principal Investigator, that the investigators are qualified to conduct the research, and that there are adequate resources (including financial, support and facilities) available. I support this application, and hereby submit it for further review.

_____ _____

Signature of Department Chair or designee Date

_____ _____

Print Name of Department Chair or designee Department

Part A-2. Summary Checklist

Are the following involved?	Yes	No
A-2 1. Existing data, research records, patient records, and/or human biological specimens?	☐	☐
A-2 2. Surveys, questionnaires, interviews, or focus groups with subjects?	☐	☐
A-2 3. Videotaping, audiotaping, filming of subjects?	☐	☐
A-2 4. Do you plan to enroll subjects from these vulnerable or select populations:		
a. UNC-CH students or UNC-CH staff?	☐	☐
b. Non-English-speaking?	☐	☐
c. Decisionally impaired?	☐	☐
d. Patients?	☐	☐
e. Prisoners, parolees and other convicted offenders?	☐	☐
f. Pregnant women?	☐	☐
g. Minors (less than 18 years)? **If yes**, give age range: to years	☐	☐
A-2 5. a. Is this a multi-site study (i.e., involves organization(s) outside UNC-CH)?	☐	☐
b. Will any of these sites be outside the United States?	☐	☐
If yes, provide contact information for the foreign IRB.		
c. Is UNC-CH the sponsor or lead coordinating center?	☐	☐
If yes, include the ***Addendum for Multi-site Studies where UNC-CH is the Lead Coordinating Center***.		
A-2 6. Will there be a data and safety monitoring committee (DSMB or DSMC)?	☐	☐
A-2 7. a. Are you collecting sensitive information such as sexual behavior, HIV status, recreational drug use, illegal behaviors, child/physical abuse, immigration status, etc?	☐	☐
b. Do you plan to obtain a federal Certificate of Confidentiality for this study?	☐	☐
A-2 8. a. Investigational drugs? (provide **IND #**)	☐	☐
b. Approved drugs for "non-FDA-approved" conditions?	☐	☐
All studies testing substances in humans must provide a letter of acknowledgement from the UNC Health Care Investigational Drug Service (IDS).		
A-2 9. Placebo(s)?	☐	☐
A-2 10. Investigational devices, instruments, machines, software? (provide **IDE #**)	☐	☐
A-2 11. Fetal tissue?	☐	☐
A-2 12. Genetic studies on subjects' specimens?	☐	☐
A-2 13. Storage of subjects' specimens for future research?	☐	☐
If yes, see instructions within the form Consent for Stored Samples.		
A-2 14. Diagnostic or therapeutic ionizing radiation, or radioactive isotopes, which subjects would not receive otherwise?	☐	☐
If yes, approval by the UNC-CH Radiation Safety Committee is required.		
A-2 15. Recombinant DNA or gene transfer to human subjects?	☐	☐
If yes, approval by the UNC-CH Institutional Biosafety Committee is required.		
A-2 16. Does this study involve UNC-CH cancer patients?	☐	☐
If yes, submit this application directly to the Oncology Protocol Review Committee.		
A-2 17. Will subjects be studied in the General Clinical Research Center (GCRC)?	☐	☐
If yes, obtain the GCRC Addendum from the GCRC and submit complete application (IRB application and Addendum) to the GCRC.		

Part A-3. Potential Conflict of Interest

The following questions apply to **all investigators and study staff involved with this research, and/or their immediate family members (spouse, dependent children, parents, significant others).** With respect to this study, will any of the study investigators or study staff or their immediate family members:

A-3 1. Have an intellectual property interest in any technology or invention used in this study, including patent rights, copyright, etc.?	☐ yes	☐ no
A-3 2. Receive support from a non-UNC source (**other than through a sponsored research agreement**) for this research study?	☐ yes	☐ no
A-3 3. Receive any form of personal compensation (**other than as specified in the budget of a sponsored research agreement**) from a Sponsor of this study, including salary, consulting fees, honoraria, royalties, equipment, gifts, etc.?	☐ yes	☐ no
3a. **If yes**, does or will that personal compensation exceed $10,000?	☐ yes	☐ no
3b. **If yes**, is that personal compensation tied to any performance within this study such as enrollment goals for the study?	☐ yes	☐ no
A-3 4. Have an ownership interest of any nature in the Sponsor or a product used in this study, including equity, stock options, etc?	☐ yes	☐ no
4a. **If yes**, does or will that interest exceed $10,000 in value or 5% equity in a publicly traded Sponsor?	☐ yes	☐ no
4b. **If yes**, does that interest include any equity interest in a non-publicly traded Sponsor?	☐ yes	☐ no
A-3 5. Hold any position with the Sponsor, including officer, employee, director, trustee, consultant, member of advisory board, etc.?	☐ yes	☐ no
A-3 6. Have a conflict of interest previously disclosed through the University's conflict of interest evaluation process that relates to this research study?	☐ yes	☐ no

If the answer is "yes" to any of the questions above, please include an explanation with this application. As with any changes to the research itself, relationships or interests that develop later should be brought to the attention of the IRB for further consideration. Please contact the Office of University Counsel for guidance or assistance regarding the University's Conflict of Interest Policy. See http://www.unc.edu/campus/policies/coi.html for the policy.

Part A-4. Questions Common to All Protocols

For all questions, if the study involves only secondary data analysis, focus on your proposed design, methods and procedures, and not those of the original study that produced the data you plan to use.

A-4 1. **Brief Summary**. Provide a *brief* non-technical description of the study, which will be used for internal and external communications regarding this research. Include purpose, methods, and participants. Typical summaries are 50-100 words.

This research will be conducted at the EPA's Human Research Facility in Chapel Hill, NC. Approximately 12 healthy volunteers, ages 18-35, will be exposed for 4 hours to clean air and 0.4 ppm chlorine while performing moderate intermittent treadmill exercise; the exposure level will be within recommended occupational exposure limits (Occupational Safety and Health Administration, National Institute of Occupational Safety and Health, and American Conference of Governmental Industrial Hygienists). We will evaluate the effects of the chlorine exposure on upper and lower respiratory tract physiologic function and cellular and molecular response. Chlorine is a respiratory irritant, and at high exposure levels a toxic chemical, that is extensively utilized in both industry and the home resulting in large numbers of individuals being at risk for exposure. Despite this widespread use, previously conducted clinical studies are few in number and our knowledge of the effects of short term exposure to low levels of chlorine on the human respiratory tract is limited. This is the first of a projected series of studies that will provide a better understanding of the human response to inhaled chlorine and thereby reduce uncertainty in regulatory decision-making

A-4 2. **Purpose and Rationale**. Provide a summary of the background information, state the research question(s), and tell why the study is needed. If a complete rationale and literature review are in an accompanying grant application or other type of proposal, only provide a brief summary here. If there is no proposal, provide a more extensive rationale and literature review.

Purpose: In this study, we plan to evaluate the upper and lower respiratory tract response of normal healthy individuals exposed to 0.4 ppm chlorine for 4 hours while performing moderate treadmill exercise. The purposes of this study are to (1) confirm that healthy, young adults show, at most, modest decrements in lung function when exposed to low levels of chlorine gas in a controlled setting and that there is no or minimal change in airway reactivity; (2) identify the effects of low level chlorine gas exposure upon respiratory tract markers of epithelial injury and inflammation; (3) characterize the time course of symptom and pulmonary function responses to chlorine exposure; (4) assess the range of individual variability in response to chlorine; and (5) provide preliminary data for the design of future studies.

Background: Chlorine is a chemically reactive gas which is poorly soluble in water but which hydrolyses rapidly to form hydrochloric acid and hypochlorous acid, both of which are also highly reactive (Winder, 2001). This rapid hydrolysis results in the ability of the nasal and oral cavities to efficiently scrub most chlorine from inhaled air. In a study in which boluses of 3 ppm chlorine were inhaled by volunteers at several inspiratory flow rates, more than 95% of inhaled chlorine was taken up in the nose, mouth, and pharynx and less than 5% was taken up in the lower airways and gas exchange regions of the lung (Nodleman and Ultman 1999). Although not certain, the prevailing hypothesis is that the effects of chlorine are primarily mediated through the actions of hypochlorous acid formed in the lining fluid of the respiratory tract (Das and Blanc, 1993). It is likely that effects are mediated both by stimulation of airway neural receptors and by damage to epithelial cells lining the airways. Hydrochloric acid is also known to have irritant and toxic properties but probably at higher concentrations than for hypochlorous acid.

Das and Blanc (1993) summarize the effects of short-term exposure from the animal literature as indicating mild focal irritation of the nose and trachea without lower respiratory effects at 2 ppm; pneumonia and bronchiolitis obliterans following acute inhalation of 50 ppm or following subacute inhalation of 9 ppm; and mortality occurring following higher concentrations. Winder (2001) estimates from the literature that cough, choking, and burning will be present in humans exposed to 15 ppm, pneumonitis will result following exposure to 50 ppm and death will occur following 30 minutes exposure to 430 ppm. Winder (2001) concludes that most workers will tolerate a time-weighted average of 1 ppm, although it is probable that some sensitive workers will show signs of irritation below this value. Withers and Lees (1985) summarize the literature as indicating that for humans 4 ppm is irritating and normal work is impossible; 16-60 ppm is dangerous (undefined) for exposures of 0.5 to 1 hour duration; 100 ppm is incapacitating and intolerable.

Long-term exposure to low levels of chlorine can induce chronic respiratory system effects as demonstrated by mild, focal lesions of the nose and trachea (epithelial hyperplasia with loss of cilia and goblet cells) observed in Rhesus monkeys following 5 day per week exposure for 1 year to 2.3 ppm chlorine (Klonne et al 1987). Epidemiologic studies of the effects of long-term, low-level occupational exposure in humans, however, provide conflicting results and are not conclusive (Das and Blanc 1993, Winder 2001). Several cross sectional studies have demonstrated an increased prevalence of bronchial hyperreactivity among competitive swimmers (Helenius et al, 1998, Zwick et al 1990). It is not clear to what extent exposure to chlorine and chloramines in the air above swimming pools contributes to these observations.

As with many other respiratory irritants (e.g. ozone, nitrogen dioxide), short-term exposures to increasing levels of chlorine (e.g. as the result of industrial accidents, tank car derailments, military use in World War I) result in increasing levels of pulmonary injury leading to airway obstruction, inflammation, pulmonary edema, and death at the highest levels (Adelson and Kaufman 1971, Das and Blanc 1993, Winder 2001). The levels and time of exposure necessary to cause severe adverse effects in humans are ill-defined primarily because measures of airborne chlorine concentrations are almost never available during accidental gassing episodes. In one case of accidental exposure for which chlorine concentrations were estimated as high as 66 ppm, 88 people were admitted to the hospital following exposure for up to 1 hr. There was no mortality reported, but many individuals had severe symptoms and physiological changes as well as airway injury noted on bronchoscopy performed five days after exposure (Shroff et al 1988). Just recently, in January 2005, a train wreck/derailment in Graniteville South Carolina released approximately 90 tons of chlorine into the atmosphere over 24 hours. Thousands of persons were evacuated, over 250 sought medical attention and 8 persons died from acute chlorine gas toxicity. The on-site and near site exposure concentrations where the deaths occurred are unkown.

The extent to which humans exposed acutely to high concentrations of chlorine experience residual effects is a matter of ongoing debate (Das and Blanc 1993, Winder 2001). Numerous case studies and epidemiologic studies addressing this issue provide conflicting information and are difficult to interpret because of the following deficiencies or confounders: (1) no quantitative estimates of concentration during accidental exposure; (2) inadequate control of effects of potential confounders (e.g. tuberculosis in early studies, smoking, concurrent long-duration low-level chlorine exposure in occupational cohorts, and potential long- and short-term co-exposure to other gases); (3) the possibility of pre-existing airway hyperreactivity or respiratory disease such as asthma; and (4) a lack of baseline lung function or bronchial reactivity measures prior to exposure. Early studies focused on persistent respiratory symptoms and decrements in forced expiratory spirometry as measures of effect. They show that of the very highly exposed (usually characterized as producing immediate severe respiratory symptoms requiring medical attention), the great majority recover without sequelae while a small proportion likely incur persistent respiratory symptoms and/or lung function decrements with or without disability.

More recent studies have addresses the question of whether a single or multiple high-concentration exposures can induce new asthma or result in a reactivation of quiescent asthma. Some cross-sectional studies have observed what appears to be a higher than expected prevalence of nonspecific bronchial hyperreactivity in occupational groups previously exposed to high chlorine concentrations (Schwartz et al

1990, Gautrin et al 1995). However because of a lack of baseline measures it is not clear whether bronchial hyperreactivity was the result of chlorine exposure or whether pre-existing bronchial reactivity in some members of the group may have resulted in more severe acute symptoms and a greater likelihood of reporting exposure. Several case studies have reported that some individuals who incurred a short-term chlorine exposure (concentration undefined) sufficient to cause acute respiratory symptoms and lung function changes subsequently developed reactive airways dysfunction syndrome (RADS) or "irritant-induced" asthma (Das and Blanc 1993, Donnelly and FitzGerald 1990, Schonhofer et al 1996, Moore and Sherman 1991, Deschamps et al 1994, Brooks et al 1985). In many of these cases, the individuals had a prior history of asthma or other chronic pulmonary disease making interpretation difficult (Donnelly and FitzGerald 1990, Moore and Sherman 1991). However, in other cases, the affected individuals were without known pre-existing respiratory disease (Schonhofer et al 1996). In one of the RADS cases reported to have occurred in relation to chlorine or chlorine dioxide exposure, bronchoscopy 60 hours after exposure demonstrated severe injury of the lower respiratory tract suggesting that the exposure had been substantial (Lemiere et al 1997). In an occupational cohort study in which baseline airway reactivity was measured prior to exposure, a relationship between an increase in airway hyperreactivity and recent accidental chlorine exposures was observed (Gautrin et al 1999). In that same study, 3 of 13 workers reporting to the workplace medical unit for evaluation of an accidental "gassing" incident were found to have had a transient but reversible increase in nonspecific airway reactivity (Leroyer et al 1998). Malo et al (1994) reported that some chlorine-exposed workers with airway hyperreactivity experience an improvement in airway reactivity over time following cessation of exposure. It thus appears that short-term, high concentration chlorine exposure may induce reactive airways dysfunction syndrome or irritant induced asthma in susceptible individuals while the great majority experiencing accidental exposure recover without sequelae; a current or previous history of asthma or other respiratory disease and possibly smoking may enhance susceptibility. The exposure conditions necessary for induction of RADS are not known, although most reported examples of chlorine-associated RADS involve exposures which immediately produced respiratory symptoms and resulted in medical evaluation and treatment; limited data suggest that those developing RADS experience substantial epithelial injury.

There are only a few published clinical studies of the effects of low level chlorine (0.1 ppm to 2.0 ppm) exposure upon human volunteers (Anglen 1981, Rottman et al 1983, D'Alessandro et al 1996, Schusterman et al 1998). The published studies have shown reversible effects including symptoms of eye, nose, and throat irritation cough, chest tightness, and shortness of breath; lung function changes indicative of both obstructive and restrictive changes; and increases in nasal resistance (Anglen 1981, Rottman et al 1983, D'Alessandro et al 1996, Schusterman et al 1998, Schinns et al 2000). No long-term sequelae of these experimental exposures were reported. Based upon a limited number of controlled human studies, there seems to be little physiological or symptom effect in healthy volunteers following exposures at or below 0.50 ppm for 4 hours duration (Rottman et al 1983, Anglen et al 1981, Schinns et al 2000). Controlled exposures conducted above this level include one with 8 healthy volunteers exposed for 8 hours to 0.0, 0.5, and 1.0 ppm while performing light intermittent exercise (Rottman et al 1983). Percent changes from pre-exposure values are given for 7 of the volunteers from that study in the following table.

Percent Change from Pre-exposure (from Rottman et al 1983)

	0.0 ppm		0.50 ppm		1.0 ppm	
	4 hr	8 hr	4 hr	8 hr	4 hr	8 hr
FVC	-2.4%	-3.0	-1.9%	-5.0%	-6.6%	-12.7%*
FEV1	-1.9%	-3.3%	-2.8%	-5.0%	-8.5%*	-17.8%*
Raw	-5.2%	6.2%	5.4%	17.1%	44.3%*	121%*

*P < 0.05 paired *t* test Raw = Airways Resistance

Changes following either 4 or 8 hour exposure to 0.5 ppm were minimal compared to clean air. Following 4 hr exposure to 1.0 ppm, subjects experienced lung function changes consistent with small obstructive and

restrictive effects while the magnitude of these effects were greater following 8 hours of exposure to 1.0 ppm. Lung function improved during the 2 hours following exposure, and 24 hours post-exposure was no different than the sham exposure. The authors noted no problems for any subjects during the follow-up period. The exposure of one subject was terminated after 4 hours exposure to 1.0 ppm chlorine due to severe wheezing, shortness of breath and large lung function decrements (FVC 43% and FEV_1 57%). Of note, this volunteer had a history of allergic rhinitis and, as detailed below, persons with nonspecific airway hyperreactivity are likely more acutely responsive to chlorine exposure.

In a study by D'Alessandro et al (1996), 10 individuals were exposed for 1 hour to 1.0 ppm chlorine breathing at a rate of 20 l/min in response to 5% CO_2 in the ambient air; endpoints included symptoms, pulmonary function, and airway reactivity. Five of these persons had pre-existing airway hyperreactivity to methacholine and 5 did not. Normal individuals experienced minimal airway obstruction whereas the group with airway hyperreactivity experienced significantly greater airway obstruction of moderate degree. Normal individuals reported no respiratory or irritant symptoms and neither group showed change in pulmonary function 24 hours post exposure. No changes in nonspecific airway reactivity were observed in both groups either immediately following or 24 hrs post exposure. Shusterman et al (1998) exposed 8 volunteers with allergic rhinitis and 8 without rhinitis to 0.5 ppm chlorine for 15 minutes, and observed a 24% increase in nasal airway resistance in the rhinitic subjects, but no change in the nonrhinitic subjects. These two studies, as well as the person with hay fever in the study by Rottman et al (1983), suggest that individuals with nonspecific airway hyperreactivity likely have enhanced sensitivity to the effects of chlorine gas exposure

The results of clinical studies as well the findings of animal, epidemiological and occupational studies provide the basis for determining occupational exposure standards. The current U.S. Occupational Safety and Health Administration (OSHA) Permissible Exposure Limit (PEL) for an 8 hour workday is 1.0 ppm as a ceiling (U.S. OSHA web site 2003). Based upon one study which observed some nasal and ocular symptoms following exposure to 0.5 ppm, OSHA has proposed (but not implemented) changing the PEL to a 0.5 ppm Time Weighted Average (TWA) for 8 hours with allowance for 15 minute excursions to 1.0 ppm. The current National Institute of Occupational Safety and Health (NIOSH) Recommended Exposure Level (REL) for chlorine is 0.5 ppm as a ceiling for a 10-hour workday (NIOSH web site 2003). The American Conference of Governmental Industrial Hygienists (ACGIH) has assigned chlorine a TLV of 0.5 ppm for an 8-hour workday and a STEL of 1.0 ppm for periods not to exceed 15 minutes. The ACGIH recommends that exposures at the STEL concentrations should not exceed 4 per day with each separated by at least 60 minutes (OSHA web site 2003). The Immediately Dangerous to Life and Health (IDLH) level is 10.0 ppm (NIOSH web site 2003). According to Winder (2001), "With considerable consistency around the world, chlorine has a time-weighted average exposure standard of 0.5 to 1.0 ppm, and where recommendations exist, a short-term exposure limit of 1 to 3 ppm." A four-hour exposure to the concentration in our proposed study (0.4 ppm) would have an eight-hour TWA of 0.20 ppm and would be within the limits recommended by these agencies.

Rationale: It is anticipated that this study will be the first in a series of studies to more completely elucidate the acute effects of short-term, low-level chlorine gas (and possibly other respiratory irritants) exposure upon the respiratory system. The questions of interest and the research designs of this and future studies are heavily influenced by our extensive experience studying the effects of ozone, another respiratory irritant gas. It is anticipated that some similarities in response exist among irritant gases in general and that understanding the basis of similarities and differences among gases with specific chemical properties will advance our knowledge of health effects of a larger number of respiratory irritants. We had originally considered as a first study a concentration-response study of lung function and respiratory symptoms in which volunteers would have been exposed to several levels of chlorine gas. Formal extramural scientific reviews (attached) by Paul Blanc, MD, an occupational health expert with considerable research experience with chlorine gas exposure, and by Colin Solomon, PhD, an investigator currently conducting controlled exposures of humans to chlorine gas, both suggested that we should first document that exposure to a single low level of chlorine can be conducted safely and without evidence of

substantial respiratory tract injury before proceeding with exposure to higher concentrations. This current protocol reflects that advice with the proposed chlorine exposure being within the NIOSH recommendations and including assessment of acute pulmonary injury through the procedure of bronchoalveolar lavage (BAL).

We are interested in studying the respiratory responses to low-level chlorine exposure for several reasons. First, large numbers of individuals are at risk for exposure to harmful concentrations of chlorine gas. Chlorine gas is widely used in industry and outside the workplace and is transported in large quantities throughout the world. Industrial use (production of chlorinated organic chemicals, bleaching in the pulp and paper industries, chlorination of drinking water, etc.) results in long-term, low-level exposure and occasional high concentration, short-term exposure of employees in these industries. Approximately 25 billion pounds of chlorine are produced annually in the U.S. and in 1983, an estimated 191,000 U.S. workers were at risk of exposure to chlorine in some form (Das and Blanc, 1993). Outside of industry, common exposures include swimming in, and maintenance of, chlorinated pools and prolonged exposure to inappropriate mixing of cleaning agents (e.g. bleach with either an acid or ammonia) which generates chlorine or chloramine gas. Most inhalation exposures to chlorine which are reported to poison control centers are environmental or household rather than industrial with the largest single category of these due to mixing of cleaning agents (Blanc, 1991). Although infrequent, transportation accidents (e.g. railroad tank car derailment) or industrial accidents result in environmental exposure of large numbers of people to high and low chlorine concentrations for minutes to hours. For example in 1996, 60 tons of chlorine were released from a derailed freight train near Alberton, Montana resulting in a large percentage of evacuated residents reporting adverse health effects (Horton 2002). In Graniteville, South Carolina, thousands of persons were evacuated, over 250 sought medical attention and 8 persons died from acute chlorine gas toxicity when a train accident/derailment released approximately 90 tons of chlorine over 24 hours in January 2005. Due to both the toxicity of chlorine exposure and the large scale industrial production and use of chlorine, both the Federal Emergency Management Agency and the Department of Homeland Security have identified chlorine as a chemical of high interest with regards to both industrial accidents and as a terrorism risk. Internationally, chlorine gas accounts for the largest single cause of major toxic chemical release incidents (Das and Blanc, 1993).

Second, our understanding of the effects of short-term exposure to low levels of chlorine gas on the human respiratory tract is limited. Previously conducted studies are few in number, have focused on symptom and physiologic responses, and have for the most part utilized healthy young individuals as study subjects. There are no clinical studies that have examined the human respiratory tract response to chlorine at the molecular and cellular level, the variability in response among individuals is poorly understood, and the role of chlorine gas inhalation in exacerbation of asthma and its effects on airway reactivity is relatively unexplored. The immediate results of this study will provide better characterization of the cellular and biochemical (both epithelial and inflammatory) responses of the human upper and lower respiratory tracts to a level of chlorine exposure that is expected to induce small changes in lung function. In addition, the results of this study will provide better characterization of the respiratory physiologic and symptom effects due to low level chlorine exposure especially with regards to time-course and individual variability. Based upon the work performed by Rottman et al (1983) and correcting for differences in protocol dose rate (CxV_E), we expect that persons participating in the proposed study will incur decrements in lung function y less than those shown above in the Background section at 1 pmm for 4 hours (-8.5% FEV_1; +44% Raw). Assuming that we can confirm the results of other investigators' studies, we anticipate conducting further studies at both lower and higher concentrations to elucidate concentration-response characteristics and to further explore any cellular and biochemical responses observed in this preliminary study. Areas of further interest which may be addressed in this or other laboratories include the effects of exposure upon airway reactivity and the effects of exposure upon volunteers with mild to moderate asthma.

Finally, there are many respiratory irritant gases to which individuals are occupationally or environmentally exposed for which the U.S. EPA must estimate population risks. Due to the sheer number of these substances not all can be extensively studied in humans. Additionally, some individual pollutants cannot be studied experimentally in humans because besides their respiratory irritant effects, they may have

other untoward effects which preclude voluntary exposure of humans (e.g. formaldehyde is associated with nasal cancer). Some of the most ubiquitous of these gases such as ozone, nitrogen dioxide, and sulfur dioxide have been extensively studied and are regulated by the U.S. Environmental Protection Agency as Criteria Pollutants under one section of the Clean Air Act while others including chlorine, hydrogen chloride, hydrogen fluoride, phosgene, formaldehyde, other aldehydes, etc. are regulated as Hazardous Air Pollutants (HAPS) under another section of the Clean Air Act. In order for the U.S. EPA to estimate the risk of adverse effect due to exposure to these HAPS as required by the Clean Air Act, the relationships between exposure to each of these substances and adverse human health effects must be quantified. Where human experimental data are not available, EPA relies upon animal toxicological data, *in vitro* data, and epidemiological data to predict effects and imposes considerable safety factors in calculating acceptable concentrations to account for uncertainties inherent in extrapolating from animal or *in vitro* data and inherent in the usually poor exposure data from epidemiological studies. One long-term goal of the current line of research is to use what we do know about representative irritant gases such as ozone and chlorine to reduce the uncertainty about what we know about other irritant gases. Although the basic mechanisms by which respiratory effects are caused may vary somewhat from gas to gas, many similarities exist in that most of these compounds stimulate neural receptors in the upper and lower airways resulting in respiratory symptoms and some lung function changes; many of them cause respiratory epithelial cell injury and inflammation which may be accompanied by increased epithelial permeability and airway obstruction; and many increase nonspecific airway reactivity. Most of these gases are extremely toxic at very high concentrations and irritating at lower concentrations. Furthermore, the distribution of uptake of any gas in the respiratory tract should be a function of the chemical and physical properties of the specific gas as well as the physical characteristics of the airway, the chemical characteristics of the airway lining fluid, and the pattern of breathing. We hypothesize that there are similarities in the exposure-response relationships of many of these substances, and we intend to compare the E-R relationships for ozone with those for chlorine. Using chlorine as an example, the U.S. EPA is also evaluating new methodology for estimating the safety factors which should be used when predicting human responses from animal toxicologic data. The response data from this and later human studies will be used with chlorine uptake data from humans and animals and response data from animals to determine whether the uncertainty inherent in using animal data to predict acute human response to irritant gases can be reduced.

A-4 3. **Full description of the study design, methods and procedures.** Describe the research protocol. Discuss the study design; study procedures; sequential description of what subjects will be asked to do; assignment of subjects to various arms of the study if applicable; doses; frequency and route of administration of medication and other medical treatment if applicable; how data are to be collected (questionnaire, interview, focus group or specific procedure such as physical examination, venipuncture, etc.). Include information on who will collect data, who will conduct procedures or measurements. Indicate the number and duration of contacts with each subject; outcome measurements; and follow-up procedures. If the study involves medical treatment, distinguish standard care procedures from those that are research. If the study is a clinical trial involving patients as subjects and use of placebo control is involved, provide justification for the use of placebo controls.

Study design. This is a double-blinded randomized clinical trial in which participants will be exposed on two occasions, once to clean air and once to 0.4 ppm chlorine gas. Order of exposure will be randomized and blocked to ensure that six volunteers receive chlorine exposure first, and six receive air exposure first. The clean air and chlorine exposure days will be separated by a minimum of four weeks. Our primary study endpoints are: 1) FEV1 as measured by spirometry, and 2) lung inflammation as evaluated by bronchoalveolar lavage. The performance of these tests and procedures are considered essential to conducting the study. The methacholine inhalation challenge is considered important as a screening measure and because we intend to establish that there is a small to modest change in non-specific bronchial reactivity following low level chlorine exposure. Telemetry and oxygen saturation monitoring are

part of our safety precautions and will be performed as detailed in the protocol. All other tests, (lung volumes and airways resistance by body plethymography, N2 washout, CO diffusing capacity, nasal resistance, nasal lavage, nasal and pulmonary NO production, exhaled breath condensate, and blood analysis) are considered of interest but are not essential to the conduct of this study. The performance of these tests will be optional and at the discretion of the investigators based upon the availability of appropriate study personnel and equipment as well as time constraints.

Prior to entry into the study, all potential subjects will first (1) have been recruited and undergone medical evaluations as described in UNC IRB Protocol 95-EPA-66 "Recruitment of Subjects for EPA Studies," UNC IRB Protocol 95-EPA-96 "Recruitment of Subjects for EPA Studies-Second Stage," and UNC IRB Protocol 91-EPA-304 "Effects of In-vitro Pollutant Exposure of Functional and Biochemical Characteristics of Human Pulmonary Cells in Normal Subjects." After this pre-qualification, subjects expressing an interest in the study will visit the facility for (1) an informed consent and training session; (2) 2 exposure sessions with BAL on the following day; and (3) 1 additional session for bronchial inhalation challenge with methacholine for subjects who show increased methacholine sensitivity after the second exposure. In addition, at the start-up of the study, 2 to 4 volunteers will be asked complete a dry run; the purpose of this dry run is for training of study staff and identifying any time-management problems. This involves a chamber exposure to clean air and the associated pre/post testing except for the venipuncture and next day bronchoscopy.

1. Informed consent and training session- Each potential volunteer who meets the medical criteria will have the study explained by an investigator and will grant informed consent if interested. A pregnancy test will be conducted for females. Volunteers will be trained to perform testing of lung function, exhaled breath condensate collection (EBCC), nasal resistance, nasal lavage, and nasal and pulmonary NO production. Additionally subjects will be trained in treadmill walking and the treadmill speed and elevation which results in a minute ventilation of 30 l/min will be determined. Volunteers will undergo bronchial inhalation challenge with methacholine. ECG will be monitored by telemetry during training.

2. Exposure days and next day BAL - Vital signs and any change in medical history will be ascertained, and pregnancy tests will be conducted on females prior to each exposure. ECG electrodes will be placed for telemetry monitoring. Since bacteria in the mouth produce ammonia which reacts with chlorine thereby potentially impacting exposure level, we will request that volunteers brush their teeth prior to entering the exposure chamber (toothbrush and toothpaste supplied). All exposures will be of 4 hrs duration and will be conducted in one of EPA's Human Studies Facility controlled exposure chambers (See below). Prior to exposure, individuals will undergo testing and/or the procedure of blood draw, nasal resistance, nasal lavage, nasal and pulmonary NO production, EBCC, spirometry (FVC), lung volumes and airway resistance by plethysmography, nitrogen washout, carbon monoxide diffusing capacity, and will complete symptom questionnaires. During exposure, participants will perform 20 minutes of moderate treadmill exercise (minute ventilation to be approximately 22-30 liters/min) during each 40 minutes of exposure. Symptom questionnaires, spirometry, and acoustic rhinometry, will be completed every 40 minutes during each exposure. Minute ventilation will be measured for 2-3 minutes during each exercise period; as a safety measure, oxygen saturation by pulse oximetry will be checked just prior to the end of each exercise session.

Following exposure all of the pre-exposure measures will be repeated. Volunteers will rest in the laboratory or medical station between times of testing. Methacholine challenge will be performed approximately 90 minutes after the end of exposure.

On the morning following each exposure, volunteers will return to the facility where they will undergo all procedures and tests that were performed prior to exposure. They will then undergo bronchoscopy and bronchoalveolar lavage as described below.

3. Bronchial inhalation challenge session- Subjects who showed increased bronchial reactivity following the second exposure will return approximately 3 weeks later to undergo an exhaled breath condensate collection and a bronchial inhalation challenge with methacholine. The purpose of this follow-

up bronchial challenge is to confirm that there is no long term effect on bronchial reactivity and thus this interval may be longer if an intervening respiratory tract infection has occurred.

The following provides a schedule of subject exposure day activity:

TIME (min)	ACTIVITY	EVALUATIONS & TESTS
30	Medical station evaluation	vital signs, medical history, pregnancy test, telemetry, venipuncture, teeth brushing
90	Preexposure testing	nasal and pulmonary NO production, nasal resistance, nasal lavage, EBCC, spirometry, lung volumes and Raw by body box, N2 washout, CO diffusing capacity, symptoms
240	Chamber Exposure	
00	Begin exposure	
00-20	Rest	
20-40	Treadmill	V_E, HR, O_2 sat
40-60	Rest	FVC, nasal resistance, symptoms
60-80	Treadmill	V_E, HR, O_2 sat
80-100	Rest	FVC, nasal resistance, symptoms, lunch
100-120	Treadmill	V_E, HR, O_2 sat
120-140	Rest	FVC, nasal resistance, symptoms, lunch
140-160	Treadmill	V_E, HR, O_2 sat
160-180	Rest	FVC, nasal resistance, symptoms
180-200	Treadmill	V_E, HR, O_2 sat
200-220	Rest	FVC, nasal resistance, symptoms
220-240	Treadmill	V_E, HR, O_2 sat
90	Postexposure testing	venipuncture, repeat preexposure testing,
60	Bronchial reactivity test	methacholine challenge
30	Medical station check-out	vital signs, remove telemetry leads, discharge information

Procedure description

1. Symptom questionnaires- Volunteers will be asked to rate respiratory symptoms (e.g. nasal irritation, shortness of breath, cough, etc) and sham symptoms (e.g. sweating, fatigue, etc) as none, trace, mild, moderate, severe.

2. Lung function testing- Forced expiratory spirometry, measurement of lung volume and airway resistance by plethysmography, N2 washout, and CO diffusing capacity are all standard, noninvasive, clinical tests performed on the same commercially available equipment as is used in hospital pulmonary function laboratories. Complete descriptions of each test are included in a currently approved protocol 01-EPA-249.

3. Measurement of nasal resistance- Concurrent with the lung function measurements (pre, during, and post exposure) on the two exposure days, the internal cross-sectional area and volume of the nose will be measured by acoustic rhinometry (Hilberg et al, 1989). Nasal resistance is calculated from this data. This is a non-invasive technique that utilizes the reflection of sound waves emitted into the nose via a nosepiece. Subjects will be trained in the proper placement of the nosepiece and will be able to self-administer the test during the exposures.

4. Nasal lavage- after observing a demonstration of this technique, the volunteer will spray a total of 4 milliliters of saline into each nostril using a hand held nebulizer that delivers 100 microliter/actuation (spray). Each lavage consists of eight sets of five sprays. The volunteer will blow his/her nose into a specimen cup immediately after each set of five sprays. The entire procedure should be completed in approximately 10 minutes. Visible plugs will then be manually selected from the sample and treated with Dithriothreiotol (DTT, 0.1%, 15min.vol(ml)= 4x plug wt.) then washed with BPBS (5min.same vol as DTT). Following filtration (52micron pore), total cell count and cell viability (Trypan Blue exclusion staining) is performed, then cytospin slides are made for differential leukocyte counts (Wright stain). The fluid phase is frozen at -80 deg C for future analysis. Nasal lavage measurements will include, differential cell counts, soluble or cell markers of mucosal injury, inflammation and oxidative stress (e.g. cytokines, urate levels, LDH, fibronectin), functional assays, and metal homeostasis.

5. Pulse oximetry- A finger will be placed in the sensing unit for 1 minute for measurement of arterial O_2 saturation.

6. Bronchial inhalation challenge- This test measures changes in lung function (FEV_1) resulting from inhalation of a metered amount of bronchoconstrictor aerosol and provides a measure of airway reactivity or sensitivity. Bronchoprovocation testing with methacholine is commonly used in clinical medicine for diagnosis of asthma and our methodology conforms with recommendations of the American Thoracic Society (Crapo et al 2000). The methacholine aerosols are generated using a Jet Nebulizer from solutions of the drug dissolved in normal saline. The bronchoconstricting aerosol is added throughout the first second of inhalation while the subject inhales through an aerosol triggering device; subjects inhale five breaths containing the aerosol. Two minutes after the start of aerosol inhalation, subjects perform one or two forced expiratory volume maneuvers. This sequence of aerosol inhalation followed by forced expiratory spirometry is repeated using progressively increasing concentrations of the methacholine solution.

Methacholine concentrations will be 0.00 (saline control), 0.39, 0.78, 1.56, 3.12, 6.25, 12.5, and 25 mg/ml for testing following exposure sessions; the two lowest doses will be skipped during the training session. The testing will be terminated once the subject's FEV_1 is decreased by at least 20% of the baseline value. If the target FEV_1 decrement is not attained after the 5 breaths of 25 mg/ml solution generated aerosol, the subjects will inhale 10 breaths of the 25mg/ml aerosol. The concentration (provocative concentration) of methacholine required to produce a 20% FEV_1 decrement provides a valid assessment of the subject's airway reactivity. In our recent studies, this provocative concentration for asthmatic subjects has generally been less than 8.0 mg/ml, and nonasthmatic volunteers usually do not experience the FEV_1 decrement at concentrations less than 10 mg/ml. Potential subjects will be excluded from participation in the study if their provocative concentration is less than 10 mg/ml during the training session. Testing will be terminated if in the judgment of the investigator an unwarranted degree of discomfort, risk, or anxiety is present or if the subject asks to discontinue for any reason. The bronchoconstricting effects of methacholine

begin dissipating immediately and are substantially relieved within 30 to 60 min of drug inhalation. The effects are also readily reversible with bronchodilators, such as inhaled albuterol which will be offered to the subjects if needed. There are no systemic effects of methacholine.

7. Treadmill exercise- Volunteers will walk on a motorized treadmill for 20 minutes of each 40 minute period at a speed and rate of incline determined to result in a minute ventilation of approximately 15 l/min per meter squared body surface area (BSA) with a maximum of 30 liters/min. Since the body surface of the average size male is about 2 liters, most males will exercise at the 30 liter/min rate; most female participants will exercise at an intensity to elicit a ventilation of 22-30 liters/min.

8. Venipuncture- The medical station staff will draw up to 80 ml of blood from each volunteer before exposure, immediately after exposure and 18 hours after the exposure with a total volume of no more than 240 ml over the 24 hour period.

9. Bronchoalveolar Lavage - Subjects will undergo fiberoptic bronchoscopy with BAL and endobronchial brush biopsy approximately 18 hours after exposure. Details of the BAL procedures are attached as an appendix at the end of the protocol. A licensed physician who is board certified in pulmonary medicine and is experienced in the use of a fiberoptic bronchoscope will perform the procedure. BAL measurements will include, but not be limited to, differential cell counts and soluble markers of lung injury, inflammation and oxidative stress (e.g. cytokines, urate levels, fibronectin), functional assays, and metal homeostasis. Epithelial cells removed by brushing will be analyzed for changes in expression of inflammatory genes and other genes indicative of pulmonary injury or response to chlorine.

10. Chamber exposure- Exposures will be conducted in a stainless steel exposure chamber (approximately 3 x 3 x 4 m) in room 65 of the EPA Human Studies Facility on the UNC campus in Chapel Hill. Temperature and relative humidity will be maintained at approximately 72°F and 40%, respectively. Either clean air or clean air with the appropriate chlorine concentration will be established in the chamber prior to entry and will be maintained at the appropriate level during exposure. Following chamber entry, volunteers will alternate resting in a chair and exercising on a treadmill at 20 minute intervals for a period of 4 hours. Spirometry, nasal acoustic rhinometry, and symptom questionnaires will be completed every 40 minutes during exposure and minute ventilation will be measured for 3 minutes during each exercise period. Closed circuit tv monitors and an open microphone as well as a window into the chamber will be used for monitoring and communicating with the volunteer. ECG will be monitored at all times. Water and snacks will be provided during exposure.

Exposure atmospheres will be generated by mixing chlorine gas from a cylinder of compressed gas into the air stream (cleaned and conditioned ambient air) which enters the exposure chamber from the ceiling and which exits through a perforated floor. Chlorine concentrations in the chamber will be monitored continuously using a Molecular Analytics chlorine analyzer and controlled by a computer system which compares the actual concentration in the chamber to the target concentration and regulates the amount of chlorine released from the cylinder. It is our goal and expectation that the chlorine level will be maintained within 10% of the 0.4 ppm target. Should the chamber concentration exceed the target concentration by 10%, an alarm will sound, and if reaches 0.5 ppm, chlorine flow will automatically cease and the volunteer will immediately exit the chamber.

Prior to beginning subject exposures, TRC, EPA's operations and maintenance contractor, discovered and subsequently confirmed with the instrument manufacturer that the response of the Molecular Analytics chlorine analyzers exhibits ambient pressure dependence. As ambient pressure moves away from the atmospheric pressure at the time of calibration, instrument error becomes greater and underestimates the actual chlorine concentration. To minimize this effect, TRC will perform a manual analyzer zero calibration, and a multi-point span calibration both before and directly after the exposure. We will also monitor barometric pressure during the exposure and will terminate the exposure if ambient air pressure changes by more than 10 Torr during the 4 hour chlorine exposure; a 10 Torr change would result in approximately a 7% instrument error. We expect this degree of weather change unlikely to occur and would require a major weather front to move across our location during the exposure. If an exposure is

terminated, the decision of whether the subject would continue with the study will be at the discretion of the investigator; a 3 hour 55 minute exposure would be considered differently than a 2 hr 10 minute exposure. We will not ask or allow subjects to repeat a chlorine exposure. These precautionary measures should enhance both the scientific validity of our study and subject safety.

11. Measurement of nasal and pulmonary nitric oxide production- Nasal production of NO will be measured directly by inserting an NO sampling probe into one nostril with the other nostril open to ambient air. The sampling probe consists of approximately 10 cm long tube with one end pushed flush into a compressible semi-soft olive selected for size to fit snugly into subject's nostril and the other end connected to a small filter. The disposable probe is connected to a NO sampling line and the analyzer. After inserting the olive into one of the nostrils, the subject is asked to inhale deeply and subsequently exhale against a resistor. This maneuver closes the soft palate to prevent cross-contamination of nasal sample by air from the pharynx. The measurement is terminated once NO concentration reaches a plateau lasting for at least 10 seconds, usually after 20-40 seconds of acquisition time. The measurement is repeated two to three times for each nostril (n=4-6 measurements per subject). The final data for each subject represent the mean of the acquired measurements, reported as a steady state "production" in nl/min calculated as the product of the sampling rate (0.5 ml/min) and the concentration of NO in parts per billion (ppb).

Lower airway NO will be measured by using a collection device developed in our laboratory. It is essentially a semi-automated collection technique of a method recommended by European Respiratory Society (Kharitonov et al, 1997). During a maneuver, subject connected to a mouthpiece on a device exhales slowly from a deep inspiration. After exhalation of an initial ~200 ml of air (dead space) which is discarded, the subsequent exhaled gas is collected in a Tedlar bag. During the collection phase, subject exhales for 20-30 sec. at a steady flow rate against a high-grade resistance (which elevates intraoral pressure and closes the velum). Since variations in expiration rate will affect the NO measurement the uniformity of a flow rate is displayed on a LED assembly for a feedback to subject. This procedure will be repeated at three to four different flow rates. By calculating the volume collected or exhaled and the time for collection, and knowing the expiratory flow rate, the sampling rate of the NO analyzer and NO concentration, lower airway production levels of NO can be calculated.

12. Exhaled breath condensate collection – For the collection of exhaled breath condensate, subjects will breath at normal tidal volume and frequency for 15 minutes into Tygon tubing, most of which will be submerged in a bucket containing an ice-saltwater mix to condense the water vapor. Substances found in exhaled breath will be examined for indicators of lipid peroxidation and inflammation (e.g., leukotrienes, prostaglandins, and cytokines).

A-4 4. **Benefits to subjects and/or society.** Describe any potential for direct benefit to individual subjects, as well as the benefit to society based on scientific knowledge to be gained; these should be clearly distinguished. Consider the nature, magnitude, and likelihood of any direct benefit to subjects. If there is no direct benefit to the individual subject, say so here and in the consent form (if there is a consent form). Do not list monetary payment or other compensation as a benefit.

Other than the results of a physical exam and blood screening tests, there are no direct benefits to the individual volunteers. Benefits to society include a better understanding of the effects of chlorine gas on exposed individuals. The results of this study will also provide a basis for additional work to examine exposure level response relationships and the effects of chlorine exposure upon potential sensitive subpopulations. Chlorine gas is identified as an air toxic by the U.S. Environmental Protection Agency and is regulated under the Hazardous Air Pollutants (HAPS) section of the Clean Air Act. Results of this study may ultimately play a role in regulation or standard setting for occupational or environmental exposure to chlorine and other respiratory irritants.

A-4 5. **Full description of risks and measures to minimize risks.** Include risk of psychosocial harm (e.g., emotional distress, embarrassment, breach of confidentiality), economic harm (e.g., loss of employment or insurability, loss of professional standing or reputation, loss of standing within the community) and legal jeopardy (e.g., disclosure of illegal activity or negligence), as well as known side effects of study medication, if applicable, and risk of pain and physical injury. Describe what will be done to minimize these risks. Describe procedures for follow-up, when necessary, such as when subjects are found to be in need of medical or psychological referral. If there is no direct interaction with subjects, and risk is limited to breach of confidentiality (e.g., for existing data), state this.

Chlorine-induced symptoms/lung function changes It is likely that the majority of participants exposed to 0.4 ppm chlorine will experience mild symptoms of eye, nose, and throat irritation, cough, and possibly shortness of breath. They may show small decrements in lung function (e.g. FEV_1 and FVC decrements smaller than 8%, and possible increases in residual volume) which will begin to improve on cessation of exposure and should be completely resolved within 24-48 hours. The most responsive individuals may have somewhat larger effects. Mean effects are expected to fall between the mean responses of the 0.5 ppm and the 1.0 ppm exposures for the 4 hour exposures of the Rottman study in the table above. Note that the mean level of minute ventilation in this study (15 l/min per meter squared body surface area (BSA) with a maximum of 30 liters/min.) will be higher than in the Rottman study (12.5 l/min per meter squared BSA). Thus we expect the responses to 0.4 ppm chlorine in our study to be close to those observed for the 0.5 ppm exposures in the Rottman study. Subjects in this study (Rottman et al) showed a 3% decrement in FEV1 after 4 hours of exposure to 05 ppm chlorine, but this was not statistically significant when compared to the air exposure day. In the Rottman study, one volunteer with a history of allergic rhinitis experienced a 55% decrement in FEV_1 with wheezing and shortness of breath when exposed to 1 ppm for 4 hours, and it has been observed elsewhere (D'Alessandro) that individuals with bronchial hyperreactivity may be more responsive to chlorine. For the current study, volunteers with any history of asthma and volunteers with active allergic rhinitis will be excluded as well as those with hyperreactivity to methacholine challenge as measured in our laboratory. Exposures will not be conducted within 6 weeks of resolution of a respiratory infection (based upon clinical history). Exposures will be conducted by an investigator in direct contact with the volunteer, and lung function, oxygen saturation, and symptoms will be assessed every 40 minutes during exposure. At a minimum, the exposures for the initial 3 subjects will be conducted by a physician. The exposure will be terminated for any rapid change in symptoms, lung function and arterial oxygen saturation, or for any distress of concern to the volunteer or the physician. Medication and medical staff will be immediately available (within the building) for treatment of symptomatic bronchospasm or other problems should it be necessary.

Chlorine-induced respiratory epithelial injury It is likely that mild injury to the nasal epithelium will occur accompanied by inflammation following 0.4 ppm exposure. It is possible that mild epithelial injury and inflammation will occur in the lower airways. Although exposure to very high concentrations of chlorine results in pulmonary injury causing edema and death, it is extremely unlikely that severe lower respiratory tract injury and sequelae will occur given that the exposure (0.4 ppm for 4 hours) of this study is within the levels recommended by OSHA, NIOSH, and the ACGIH for occupational exposures, is well below levels considered by OSHA to be immediately dangerous to life and health (10 ppm), and is below levels used in two previously conducted human studies (1 ppm for 8 hrs, and 2 ppm for 4 hrs) which did not report effects indicative of significant pulmonary epithelial injury.

Chlorine-induced airway hyperreactivity Although there are case studies and some epidemiological evidence that short-term accidental exposures to (presumably) high concentrations of chlorine and possibly long-term exposures to lower levels are associated with airway hyperreactivity, asthma, or reactive airways dysfunction syndrome, the vast majority of individuals experiencing accidental "gassing" incidents do not experience such effects. The incidence and persistence of these effects and the conditions (concentrations, durations) under which they occur are unknown. Experimental exposure to 1.0 ppm for 1 hour with a minute ventilation of 20 l/min was found to not increase next day nonspecific airway

hyperreactivity (D'Alessandro et al 1996). There is no published literature indicating that a single four-hour exposure to 0.5 ppm would increase the risk of developing asthma in healthy volunteers without a clinical history of asthma or evidence of airway hyperreactivity. Thus, we think that it is extremely unlikely that exposures of the magnitude of this study will result in such effects. The first participant in this study (IRB study # 05-299) underwent exposure to 0.5 ppm chlorine exposure for 4 hours This volunteer reported mild symptoms, showed the expected small decrement in pulmonary function, had a robust BAL neutophilia, and showed a greater than 4 dose change is methachloline sensitivity. Our primary concern with regards to this volunteer was the magnitude of the increased methacholine sensitivity. In an amendment we decreased the exposure concentration to 0.3 ppm chlorine and altered the exercise protocol from the target VE of 30 liters/minute to 15 liters/minutes per meter BSA with a maximum of 30 liters/minute. Since the reduction in exposure level, two individuals have undergone exposure for 4 hours to 0.3 ppm chlorine. They both reported mild symptoms, showed no change in pulmonary function, had a modest BAL neutrophilia, and showed no change in response to methacholine challenge. We have since again amended the protocol to increase the chlorine exposure level from 0.3 ppm to 0.4 ppm. With the increase in exposure level, we anticipate observing mild symptoms, modest BAL neutrophila, an increase in methacholine sensitivity of one to two doses, and with enough study participants, possibly a small decrement in pulmonary function. Of note, the subject exposed to 0.5ppm chlorine has returned for her clean air exposure and her methacholine sensitivity has returned to the level observed during her training session.

Exercise at a level to produce a minute ventilation of 30 L/min should have no adverse effect although possible risks include the unlikely possibility of falling off of the treadmill which is reduced by proper training, and the rare possibility of sudden death, cardiac arrhythmia, or myocardial infarction in someone with pre-existing heart disease. This latter risk is reduced by studying only young, healthy, nonsmoking volunteers with no major risk factors for early cardiovascular disease. Individuals receive a screening medical history, physical examination, and blood work prior to participation, and during exercise, heart rate and ECG rhythm strip are monitored by telemetry.

Methacholine bronchial challenge is commonly used in clinical medicine for diagnosis of asthma and is frequently performed by nurses or technicians with physician supervision. Our laboratory has safely performed over 200 bronchial challenges with methacholine in asthmatic subjects and a larger number in nonasthmatic subjects. Our challenge methods conform to published guidelines of the American Thoracic Society (Crapo, 2000). As the challenge is controlled induction of bronchoconstriction, it entails the risk of bronchospasm. However, the technique employs small, stepwise increases in bronchoconstriction, monitored by measurement of FEV_1, and thereby is carefully controlled. Challenge will be terminated when the subject's FEV_1 falls to 80% of the baseline value, or when the highest dose (10 breaths of 25 mg/ml aerosol) of methacholine is reached. In addition, the investigator has visual and voice contact with the subject at all times and will closely observe the subject and his FEV_1 for signs of bronchoconstriction or pulmonary discomfort. The challenge testing will be terminated if an unwarranted degree of discomfort or anxiety is present. The subject may terminate the challenge at any time for any reason. The bronchoconstriction usually dissipates within 30 to 60 minutes. The effects are also readily reversible with bronchodilators, such as albuterol, which will be available under the direction of a nurse, physician's assistant, nurse practitioner, or physician. There are no systemic effects of methacholine.

Bronchoalveolar lavage and endobronchial cytology brushing may be associated with fever, pneumonia, respiratory distress, bleeding, pneumothorax or even death. These risks are explained to the subject in full detail. Bronchoscopy procedures have been continuously performed at the Human Studies Division on the UNC-CH campus for over 10 years. During this time, more than 1000 bronchoalveolar lavages have been performed without a serious incident. Established guidelines for performing bronchoalveolar lavage and brush biopsy ensure that the safety of the subject is given absolute priority.

Venipuncture risks include the possibilities of syncope, hematoma formation, or infection, although these are unlikely and are minimized by performance of venipuncture by trained personnel.

Lung function/nasal resistance measures risks are negligible.

Nasal lavage is a mildly unpleasant procedure, but the risks are neglible.

Pulmonary and nasal NO production measures risks are negligible
Exhaled breath condensate collection risks are neglible

A-4 6. **Data analysis.** Tell how the qualitative and/or quantitative data will be analyzed. Explain how the sample size is sufficient to achieve the study aims. This might include a formal power calculation or explanation of why a small sample is sufficient (e.g., qualitative research, pilot studies).

The research objective of this study is to establish that, in our laboratory, healthy volunteers can be safely exposed to low levels of chlorine gas and that they will show modest increases in BAL markers of lower respiratory tract inflammation and modest decrements in lung function. The study will follow a randomized, repeated measures design with each subject exposed for four hours to clean air and 0.4ppm chlorine on two separate occasions. The primary outcome for respiratory tract inflammation will compare the percentage of neutrophils observed in the bronchoalveolar lavage fluid obtained 18 hours after the clean air and chlorine exposures. Previous studies utilizing BAL indicate that this data will be normally distributed and therefore the appropriate parametric test (repeated measures ANOVA, paired t test) will be applied. A p value of 0.05 or less will be considered significant. We anticipate that the proposed sample size will provide adequate (80%) power for detecting a 50% increase in the percent of BAL neutrophils, based upon previous studies showing an expected mean of 1.6% with a standard deviation of 1.0. An N of 12 will provide a β of 0.01 for detecting a 100% increase in neutrophils. In evaluating effects of chlorine exposure upon lung function, the difference between the pre-exposure and post-exposure FEV_1 will be calculated and the pre-post differences for the air and chlorine exposures will be compared utilizing the paired t-test. Past studies examining pre-post FEV_1 show that the data will be normally distributed with an air day mean of 30 to 50 cc and standard deviation of 100 to 200. Applying power analysis to these values with an N of 12 and an FEV_1 decrement of 5% (225 cc) produces a worse case β of 0.09. All other data generated from this study will be analyzed on an exploratory basis.

A-4 7. **Will the data you collect or receive include any of the identifiers on the following list?**

☐ No ☐ Yes *If yes, check all that apply*:

a. ☐ Names
b. ☐ Telephone numbers
c. ☐ Any elements of dates (other than year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death. For ages over 89: all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 and older
d. ☐ Any geographic subdivisions smaller than a State, including street address, city, county, precinct, zip code and their equivalent geocodes, except for the initial three digits of a zip code
e. ☐ Fax numbers
f. ☐ Electronic mail addresses

g. ☐ Social security numbers
h. ☐ Medical record numbers
i. ☐ Health plan beneficiary numbers
j. ☐ Account numbers
k. ☐ Certificate/license numbers
l. ☐ Vehicle identifiers and serial numbers (VIN), including license plate numbers
m. ☐ Device identifiers and serial numbers (e.g., implanted medical device)
n. ☐ Web universal resource locators (URLs)
o. ☐ Internet protocol (IP) address numbers
p. ☐ Biometric identifiers, including finger and voice prints
q. ☐ Full face photographic images and any comparable images
r. ☐ Any other unique identifying number, characteristic or code, other than dummy identifiers that are not derived from actual identifiers and for which the re-identification key is maintained by the health care provider and not disclosed to the researcher

A-4 8. **Data sharing.** With whom will *identifiable* (contains any of the 18 identifiers listed in question 7 above) data be shared outside the immediate research team? For each, explain confidentiality measures. Include data use agreements, if any.

☐ No one
☐ Coordinating Center:
☐ Statisticians:
☐ Consultants:
☐ Other researchers:
☐ Registries:
☐ Sponsors:
☐ External labs for additional testing:
☐ Journals:
☐ Publicly available dataset:
☐ Other:

A-4 9. **Confidentiality of the data**. Describe procedures for maintaining confidentiality of the data you will collect or will receive. Describe how you will protect the data from access by those not authorized. How will data be transmitted among research personnel? Where relevant, discuss the potential for deductive disclosure (i.e., directly identifying subjects from a combination of indirect IDs). Describe your plan to destroy identifiers. When will identifiers be destroyed?

All subjects will be assigned an ID number; any reference to an individual will be made using that number and not the name of the subject. Names of subjects associated with ID numbers will be archived and locked; only medical and scientific personnel associated with this study will have access to this information. No personal identifying information will be attached and/or recorded in the data log sheets,

biologic samples, or electronic data sets. No subjects will be identified in any report or publication about this study. Study samples will be stored in a secure room with restricted access. The sample will be prepared and stored indefinitely in a freezer for future testing. Portions of the samples may be shared with researchers at other scientific institutions, however, only coded samples will be sent. Under no circumstances will any identifying information be sent along with samples to outside investigators. All medical records generated during this study will be kept in the medical records office at the EPA Human Studies Facility.

A-4 10. **Data security for storage and transmission**. Please check all that apply.

For electronic data:

- ☐ Secure network ☐ Password access ☐ Encryption
- ☐ Other (describe):
- ☐ Portable storage (e.g., laptop computer, flash drive)
  Describe how data will be protected for any portable device:

For hardcopy data (including human biological specimens, CDs, tapes, etc.):

- ☐ Data de-identified by research team (stripped of the 18 identifiers listed in question 7 above)
- ☐ Locked suite or office
- ☐ Locked cabinet
- ☐ Data coded by research team with a master list secured and kept separately
- ☐ Other (describe):

Part A-5. The Consent Process and Consent Documentation (including Waivers)

The standard consent process is for all subjects to sign a document containing all the elements of informed consent, as specified in the federal regulations. Some or all of the elements of consent, including signatures, may be altered or waived under certain circumstances.

- If you will obtain consent in any manner, complete **section 1**.
- If you are obtaining consent, but requesting a waiver of the requirement for a signed consent document, complete **section 2**.
- If you are requesting a waiver of any or all of the elements of consent, complete **section 3**.

You may need to complete more than one section. For example, if you are conducting a phone survey with verbal consent, complete sections 1, 2, and possibly 3.

A-5 1. **Describe the process of obtaining informed consent from subjects**. If children will be enrolled as subjects, describe the provisions for obtaining parental permission and assent of the child. If decisionally impaired adults are to be enrolled, describe the provision for obtaining surrogate consent from a legally authorized representative (LAR). If non-English speaking people will be enrolled, explain how consent in the native language will be obtained. Address both written translation of the consent and the

availability of oral interpretation. *If you are not requesting a waiver of any type, you are done with Part A-5; proceed to Part B.*

Before being selected as a participant, all volunteers will be required to read and sign a form asserting their understanding of the following: (1) participation is strictly voluntary, (2) the purpose of the study, (3) the nature and extend of participation, (4) the participant's rights to withdraw at any time, (5) the right to privacy, (6) the risks associated with participation, (7) the method of compensation, and (8) the limits of the University and investigator's liability.

An investigator will describe the study and answer any questions that may arise regarding participation, safety, issues related to payment, and any other details pertinent to the study. The investigator will then review the consent form before he/she and the participant sign it. One signed copy of the written informed consent will be given to the participant while the investigators retain the original. Study participants will have the opportunity to ask questions at any time during the study by contacting one of the PIs and the medical staff.

Participants must be fluent in English, as the EPA Human Studies Facility does not employ language translators necessary to ensure participant comprehension for those who do not fully understand English.

A-5 2. **Justification for a waiver of *written* (i.e., signed) consent**. *The default is for subjects to sign a written document that contains all the elements of informed consent.* Under limited circumstances, the requirement for a signed consent form may be waived by the IRB if either of the following is true:

a. The only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality ☐ yes ☐ no

Explain.

b. The research presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context. ☐ yes ☐ no

Explain.

If you checked "yes" to either, will consent be oral? Will you give out a fact sheet? Use an online consent form, or include information as part of the survey itself, etc?

A-5 3. **Justification for a full or partial waiver of consent.** *The default is for subjects to sign a written document that contains all the elements of informed consent.* A waiver might be requested for research involving only existing data or human biological specimens (see also Part C). More rarely, it might be requested when the research design requires withholding some study details at the outset (e.g., behavioral research involving deception). In limited circumstances, parental permission may be waived. This section should also be completed for a waiver of HIPAA authorization if research involves Protected Health Information (PHI) subject to HIPAA regulation, such as patient records.

☐ Requesting **waiver of some elements** (specify; see SOP 28 on the IRB web site):
☐ Requesting **waiver of consent entirely**

If you check either of the boxes above, answer items a-f.. To justify a full waiver of the requirement for informed consent, you must be able to answer "yes" (or "not applicable" for question c) to items a-f. **Insert brief explanations that support your answers.**

a. Will the research involve no greater than minimal risk to subjects or to their privacy? ☐ yes ☐ no
Explain.

b. Is it true that the waiver will *not* adversely affect the rights and welfare of subjects? *(Consider the right of privacy and possible risk of breach of confidentiality in light of the information you wish to gather.)* ☐ yes ☐ no
Explain.

c. When applicable to your study, do you have plans to provide subjects with pertinent information after their participation is over? *(e.g., Will you provide details withheld during consent, or tell subjects if you found information with direct clinical relevance? This may be an uncommon scenario.)* ☐ yes ☐ not applicable
Explain.

d. Would the research be impracticable without the waiver? *(If you checked "yes," explain how the requirement to obtain consent would make the research impracticable, e.g., are most of the subjects lost to follow-up or deceased?).* ☐ yes ☐ no
Explain.

e. Is the risk to privacy reasonable in relation to benefits to be gained or the importance of the knowledge to be gained? ☐ yes ☐ no
Explain.

If you are accessing patient records for this research, you must also be able to answer "yes" to item f to justify a waiver of HIPAA authorization from the subjects.

f. Would the research be impracticable if you could not record (or use) Protected Health Information (PHI)? *(If you checked "yes," explain how* not *recording or using PHI would make the research impracticable).* ☐ yes ☐ no
Explain.

Part B. Questions for Protocols that Involve Direct Interaction with Human Subjects

B 1. **Subjects.** Specify number, gender, ethnicity, race, and age. Specify whether subjects are healthy volunteers or patients. If patients, specify any relevant disease or condition and indicate how potential subjects will be identified.

Our statistical power analysis shows that we will need 12 subjects to undergo exposure to clean air and 0.4 ppm chlorine. Healthy non-smoking individuals, ages 18-35 yrs, of either gender or any ethnicity will serve as volunteers. Taking into account the three subjects who were exposed 0.3 or 0.5 ppm chlorine, subjects who fail the screening methacholine test, and subject withdrawals, we anticipate that we will need to consent 22 subjects to complete the study. Persons who use corrective lenses will be asked to wear glasses and not use their contact lenses. Pregnant women and nursing mothers will be excluded from participation as there is no evidence that they would respond any differently than non-pregnant or non-nursing women. Although we are not aware of any risk to an unborn fetus or nursing infant, we do not believe that the information to be gained by exposing pregnant or nursing women would justify any incremental risk (known or unknown) to the fetus or a nursing infant. All female participants will tested for pregnancy at the time of admission into the study and immediately prior to each exposure or bronchial inhalation challenge.

Up to four additional subjects will be recruited to participate in "dry runs" of the protocol in which no pollutants or methacholine will be administered, and no venipunctures or BAL will be performed. Exercise, acoustic rhinometry, measurement of nasal and pulmonary NO production, nasal lavage, exhaled breath condensate collection, and lung function tests will be performed as will symptom evaluation. The purpose of these is to work out any scheduling conflicts, staff training, or procedural problems prior to conducting the study. "Dry run" subjects may subsequently participate in the exposure study.

B 2. **Inclusion/exclusion criteria.** List required characteristics of potential subjects, and those that preclude enrollment. Justify exclusion of any group, especially by criteria based on gender, ethnicity, race, or age. If pregnant women are excluded, or if women who become pregnant are withdrawn, specific justification must be provided.

Inclusion criteria: Ages 18-35 yr; non-smokers; generally healthy; fluent in English; volunteers will be asked to discontinue non-steroidal anti-inflammatory drugs and any dietary supplements including multivitamins for the week prior to exposure and to avoid hot tub or pool use for 1 week prior to exposures.

Exclusion criteria:

* current or past history of asthma, other chronic pulmonary disease, or perennial rhinitis
* active allergic rhinitis (volunteers with well-defined seasonal allergic rhinitis can be studied outside of their allergy season)
* competitive swimmers
* problems with excessive bleeding after minor cuts or abrasions
* history of ocular disease and/or dry eye syndrome
* cardiovascular disease or any significant risk factors for early cardiovascular disease (as evaluated by reported exercise level, family history, physical exam, and lipid levels)

* contra-indication to moderate treadmill exercise
* anemia as identified using Lab Corp criteria

* chronic medication use other than contraceptives, low dose antibiotics for acne, stable dose of thyroid replacement hormone for three months, or vitamins/supplements
* more than 1 pack-years of cigarette smoking or any cigarette use in the past 6 months;
* FEV1/FVC <70% or nonspecific airway hyperreactivity by methacholine challenge.

Exposures will not be conducted within 6 weeks of a resolving respiratory tract infection.

B 3. **Methods of recruiting.** Describe how and where subjects will be identified and recruited. Indicate who will do the recruiting, and tell how subjects will be contacted. Describe efforts to ensure equal access to participation among women and minorities. Describe how you will protect the privacy of potential subjects during recruitment. *For prospective subjects whose status (e.g., as patient or client), condition, or contact information is not publicly available (e.g., from a phone book or public web site), the initial contact should be made with legitimate knowledge of the subjects' circumstances. Ideally, the individual with such knowledge should seek prospective subjects' permission to release names to the PI for recruitment. Alternatively, the knowledgeable individual could provide information about the study, including contact information for the investigator, so that interested prospective subjects can contact the investigator.* Provide the IRB with a copy of any document or script that will be used to obtain the patients' permission for release of names or to introduce the study. Check with your IRB for further guidance.

Volunteers will be recruited by the Westat Corporation under contract to the U.S. Environmental Protection Agency using methods which are on file with the UNC IRB (Protocol 95-EPA-66 "Recruitment of Subjects for EPA studies"). In general these normal, healthy volunteers will be recruited through newspaper advertising, posted flyers, a recruitment web site (www.epastudies.org), word of mouth, and a recruiting brochure available at the Human Studies Facility.

B 4. **Protected Health Information (PHI).** If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization*. If this applies to your study, please provide the following information.

a. Will the information collected be limited only to that necessary to contact the subjects to ask if they are interested in participating in the study?

b. How will confidentiality/privacy be protected prior to ascertaining desire to participate?

c. When and how will you destroy the contact information if an individual declines participation?

B 5. **Duration of entire study and duration of an individual subject's participation, including follow-up evaluation if applicable.** Include the number of required contacts and approximate duration of each contact.

It is anticipated that the entire study will take up to 1 year to complete. Each individual will be in the facility for 1-2 hours for medical screening, and ½ day for training. For each of the two exposures volunteers will be in the facility 1 full day on the day of exposure plus approximately 6 hours on the day following exposure. Each individual will return for approximately 2 hours for bronchial challenge testing following completion of both exposures. In the absence of illness and scheduling difficulties, each volunteer could complete the study within 8-10 weeks. In practice, it will often take longer.

B 6. **Where will the subjects be studied?** Describe locations where subjects will be studied, both on and off the UNC-CH campus.

Volunteers will be studied in the EPA Human Studies Facility on the UNC-Chapel Hill campus.

B 7. **Privacy.** Describe procedures that will ensure privacy of the subjects in this study. Examples include the setting for interviews, phone conversations, or physical examinations; communication methods or mailed materials (e.g., mailings should not indicate disease status or focus of study on the envelope).

All interviews, phone conversations, physical examinations and medical procedures (bronchoscopy) will be conducted in private rooms in the EPA Human Studies Facility. This facility is guarded and only individuals working in the building have access beyond the guard's desk without an escort.

B 8. **Inducements for participation.** Describe all inducements to participate, monetary or non-monetary. If monetary, specify the amount and schedule for payments and how this will be prorated if the subject withdraws (or is withdrawn) from the study prior to completing it. For compensation in foreign currency, provide a US$ equivalent. Provide evidence that the amount is not coercive (e.g., describe purchasing power for foreign countries). Include food or refreshments that may be provided.

Volunteers will be paid for the time that they participate ($12.00 per hour) plus additional incentives for the performance of certain procedures which produce discomfort (venipuncture $20, nasal lavage $20, bronchoscopy with BAL $350). A completion bonus of $125 will be paid for each subject who completes the study. . Subjects will be paid the same whether or not the follow-up methacholine challenge is required. A detailed break-down of the payment schedule and time requirement is as follows:

Procedure	Time	Payment
Training	4 hr	$ 48
Chamber exposure (9 hr)	18 hr	$ 216
BAL Day Testing (2hr)	4 hr	$ 48
F/U Methacholine Challenge	2 hr	$ 24
6 Venipuncture ($20 each)	N/A	$ 120
7 Nasal Lavage ($20 each)	N/A	$ 140

2 bronchoscopies ($350 each)	N/A	$ 700
2 On-time bonus ($25 each)	N/A	$ 25
1 Completion bonus ($125)	N/A	$ 125
TOTAL :		**$1471**

It is anticipated that a volunteer undergoing two exposures plus training plus follow-up with the called for venipunctures and bronchoscopies will receive a total payment of $1471. Subjects who complete the dry run at the beginning of the study will be paid an additional $148. All payment will be made at the end of the study unless a specific request for prior partial payment is made by the subject. Although a subject may choose to withdraw from the study without explanation at any time, if he chooses to end his participation in the study for non-medical reasons, he will be paid only the fees earned to that point. On the other hand, the subject will be fully paid for his time commitment if the investigators decide to terminate his participation on medical grounds or for any other reason (e.g. repeatedly late for appointments). Volunteers will be paid a nominal fee to offset transportation expenses if they travel from outside the Chapel-Carrboro area, and parking will be provided.

B 9. **Costs to be borne by subjects.** Include child care, travel, parking, clinic fees, diagnostic and laboratory studies, drugs, devices, all professional fees, etc. If there are no costs to subjects other than their time to participate, indicate this.

Participants in the study are not expected to bear any costs of the study.

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dislodged from the brush by stirring in a test tube containing tissue culture medium. Two brush biopsies will be done and will normally be obtained in the left mainstream bronchus. In some cases, the brush procedure produces some minor bleeding of the airway mucosa which, under normal circumstances, resolves in a matter of a few minutes. A 1:20,000 dilution of epinephrine can be injected through the bronchoscope at the bleeding site should it persist for more than a couple of minutes. The physician will monitor the brush biopsy sites for hemostasis before removing the bronchoscope from the airway.

Following the procedure, the subjects will be monitored for at least one hour by a nurse while they rest on a recliner. Telemetry is monitored and vital signs are checked every 30 minutes until discharge. The oxygen cannula will be removed once oxygen saturation is satisfactory. Subjects will perform post-procedure spirometry. A subject will be discharged if he/she shows no signs of complications, has a gag reflex, is able to tolerate oral intake without aspirating and has normal vital signs A physician (usually the bronchoscopist) will perform a brief physical exam and review the subject's clinical status before discharging the subject. Prior to discharge subjects will be requested to take 600mgm of ibuprofen by mouth; administration of ibuprofen almost always prevents the post bronchoscopy malaise and low grade fever that would otherwise occur in about 25% of the subjects (acetaminophen is a less effective alternative medication). Each subject will then be given a pager number and telephone numbers where he/she can reach the physician should he/she experience any side effects of the procedure.

The physician will terminate the procedure at any time if he feels that it would be injurious to the subject's well-being to continue. Examples of symptoms which will result in the termination of the procedure include: 1) Discomfort or anxiety, 2) Chest pain, 3) Tachycardia, bradycardia or other electrocardiogram abnormality, 4) Unremitting cough, 5) Moderate bronchospasm of the airways, 6) Moderate bleeding of the airways, 7) Epistaxis, 8) Arterial blood saturation of less than 93 % while on 6 l/min supplemental oxygen, 9) An adverse reaction to lidocaine. The subjects will be aware that they can terminate the procedure for any reason, at any time, and still receive full compensation for attempting it. However, if a subject or physician terminates a procedure the subject will be excluded from further participation in the study.

The bronchoscopy physician will be available by pager to deal with complications resulting from the procedure. An on-site duty physician is also available to respond. A fully-equipped emergency medical cart is available at the EPA Human Studies Division medical station. Physicians in the emergency room at UNC Memorial Hospital, located within 1/4 of a mile, are also available for treatment or consultation.

Risks of Bronchoscopy with Bronchoalveolar Lavage and Brush Biopsy:

Bronchoscopic procedures have been continuously performed at the Human Studies Division on the UNC-CH campus for over 15 years. During this time, more than 1500 bronchoalveolar lavages have been performed without a serious incident. Established protocols for bronchoalveolar lavage and brush biopsy ensure that the safety of the subject is given absolute priority. Bronchoscopic procedures are an established tool for investigational studies of asthma as well as persons with other respiratory diseases.

Bronchoscopies are performed by experienced physicians who are either Board-certified or Board-eligible in pulmonary medicine. The physician performing the bronchoscopy is always assisted by at least one nurse familiar with the procedure. The subject's vital signs and ECG are continuously monitored during the bronchoscopic procedures and at regular intervals during the recovery phase. The procedure is immediately terminated if the subject experiences any alterations such as tachypnea, depressed respiration, tachycardia, bradycardia, abnormal rhythms or significant changes in blood pressure. As indicated earlier, a fully equipped emergency cart including a defibrillator, endotracheal intubation equipment and emergency medications (atropine, epinephrine) is available at all times. An on-site physician is always available to respond and the NC Memorial Hospital Emergency Room is within 1/4 mile of Human Studies Facility. Before the subject is sent home, he/she receives a brief physical examination by a physician and is given

phone numbers and a pager number with which to contact a physician should he/she experience any problems or have any questions.

The medical screening of the subjects is specifically designed to exclude subjects with a history of medical problems which might put them at risk from the bronchoscopic procedures. Medical problems which could arise as a result of bronchoalveolar lavage and brush biopsy include the following:

Bradycardia and/or hypotension may result from increased vagal nerve output provoked by coughing or Valsalva during the passage of the bronchoscope through the vocal cords and trachea. Atropine (0.6 mg iv) may be s prophylactically administered to the subjects before the start of the procedure to minimize this risk and can be used during the procedure to block cholinergic effects. The primary side effects associated with atropine administration is mild sinus tachycardia which typically resolves within 30 minutes as the drug is cleared from the subject's system. In rare cases (less than 1 percent) the sinus tachycardia is accompanied by hypotension.

Arterial blood oxygen saturation is continuously monitored during the procedure with a finger pulse oximeter. Subjects are supplemented with oxygen at a flow rate of 2 l/min via nasal cannula during the procedure. The oxygen flow can be increased to a maximum of 6 l/min if the arterial oxygen saturation falls below 93 %. If oxygen supplementation is not effective, the procedure will be discontinued immediately. Removal of the bronchoscope and treatment with inhaled bronchodilator should be sufficient to allow the subject's arterial oxygen saturation to return to normal. Under normal conditions, oxygen supplementation is ceased once the procedure is completed. However, supplementation will be continued if arterial oxygen saturation is below 93 % on room air. In the event that low oxygen saturation persists, if the subject cannot be weaned from the oxygen supplementation, or if it falls precipitously at any time, an emergency situation will be declared and the subject will be transported immediately by ambulance to the NC Memorial Hospital Emergency Room.

Discomfort of the nose and throat is a primary risk of bronchoscopy. As outlined, the lidocaine gargle, spray and liquid is used to anesthetize these areas prior to passing the bronchoscope to reach the lower airways. The procedure will be discontinued if adequate anesthesia cannot be achieved by the means described.

Cough is a common, albeit minor, problem encountered during bronchoscopy. Cough results from mechanical stimulation of cough receptors in the airway by the bronchoscope or the cytologic brush used for the brush biopsies. In addition to the discomfort, prolonged coughing with the bronchoscope in place can result in mild mechanical trauma to the vocal cords. Lidocaine solution is passed through the bronchoscope channel at the main carina and at the level of the right mainstream bronchus to suppress the cough reflex during the procedure, more can be used as needed. The procedure will be terminated if adequate cough suppression cannot be attained.

Lidocaine use presents a small risk to the subjects, as a variable fraction of the medication is absorbed through the airway mucosa. If a significant amount of lidocaine is absorbed, adverse reactions such as bradycardia, hypotension, urticarial reactions, confusion, lightheadedness, euphoria, tremors and seizures can result. To prevent these side effects, the amount of liquid lidocaine used during the entire procedure will be limited to a maximum of 360 mg.

Bronchospasm, manifested as wheezing, chest tightness or dyspnea, is a risk of bronchoscopy that is caused by stimulation of irritant receptors in the airways by the bronchoscope. Treatment for airway constriction occurring during the procedure will be given by the physicians responsible for the procedure. If significant bronchoconstriction occurs, the procedure will be terminated. Administration of inhaled bronchodilator (albuterol) is all that is usually be required to control symptoms and improve lung function.

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Epistaxis is caused by trauma to the nose by the bronchoscope. This condition is expected to be minor and resolve on its own. Small streaks of blood in nasal secretions may be present for up to 12 hours

following the procedure. If the bleeding becomes moderate or severe during bronchoscopy, the procedure will be terminated and the bronchoscope removed. The subject's anterior nasal passage will be packed with sterile gauze to stop the bleeding. If the bleeding does not resolve with packing, the subject will be transferred to the NC Memorial Hospital Emergency Room.

Bleeding in the lower airway may occur from trauma caused by the bronchoscope or by brush biopsy. Bleeding is typically very minor, in the order of 1/10 cc, is not clinically significant in an otherwise healthy subject and should spontaneously resolve in a matter of minutes. Epinephrine, diluted 1/20,000 fold will be administered through the bronchoscope to the affected site if bleeding is minor to moderate. The maximum dose of epinephrine used will be 2-3 cc to prevent local tissue necrosis. The site will be monitored through the bronchoscope until the bleeding stops. If bleeding fails to resolve with epinephrine or if it is sufficiently severe to cause hemoptysis or hemoglobin desaturation, oxygen supplementation will be provided to keep arterial oxygen saturation above 90% and the subject will be transported to the NC Memorial Hospital Emergency Room by ambulance. If it becomes necessary, the subject can be intubated before transport.

The use of epinephrine to control bleeding can produce systemic effects resulting from mucosal absorption of the drug. These symptoms are transient and include headache, palpitations and tachycardia when the dose is greater than 1 mg and is given intravenously. Two cc of a 1/20, 000 dilution of epinephrine contains 0.10 mg of the drug, a dose that is very unlikely to induce any untoward effects. This is especially true when the drug is applied to the mucosa, since only a fraction is expected to be absorbed.

Pneumothorax is a risk of bronchoscopic procedures such as transbronchial biopsy, peripheral lung protected brushings or peripheral lung cytology brushings. Because these procedures will not be performed, the risk of pneumothorax in this study is extremely small. There is a very small risk of a pneumothorax resulting from a endobronchial brush biopsy. The symptoms include dyspnea and chest pain. The subjects will be interviewed and examined by the bronchoscopy physician 2 hours after the procedure with these symptoms in mind. If a pneumothorax is suspected, the subject will be transferred to the NC Memorial Hospital Emergency Room by ambulance for further treatment.

Low-grade fever (38-38.5 °C) can occur in approximately 25 % of hospitalized subjects undergoing bronchoscopy. The fever invariably resolves within 18 hours without treatment or with acetaminophen. Our experience at HSD, EPA has shown that this fever and the associated malaise can almost always be prevented by administering ibuprofen following the procedure. The subject will be asked to contact the physician who performed the procedure or one of the nurses at the Medical Station if fever persists or is higher than 38.5 °C. As part of the standard protocol, a nurse or physician will contact the subject by telephone between 24 and 48 hours after the procedure to inquire on the general health of the subject and specifically about the presence of fever.

The risk of pneumonia as a result of bronchoalveolar lavage in the lobe involved in the procedure is less than 1 %. Symptoms of pneumonia could include fever, dyspnea, persistent cough, productive cough and chest pain. The subject will be instructed to call the physician who performed the procedure or the Medical Station if these symptoms occur. The medical staff will evaluate the subject over the phone between 24 and 48 hours after the procedure for signs of pneumonia.

The subject will be urged to contact the Medical Station or the bronchoscopy physician should he/she experience any of the following symptoms: 1) Epistaxis, 2) Persistent cough, 3) Hemoptysis, 4) Chest pain, 5) Dyspnea, 6) Wheezing, 7) Sputum production, 8) Hoarseness or sore throat.

Part C. Questions for Protocols using Data, Records or Human Biological Specimens without Direct Contact with Subjects

C 1. What records, data or human biological specimens will you be using? *(check all that apply)*:

- ☐ Data already collected for another research study
- ☐ Data already collected for administrative purposes (e.g., Medicare data, hospital discharge data)
- ☐ Medical records (custodian may also require form, e.g., HD-974 if UNC-Health Care System)
- ☐ Electronic information from clinical database (custodian may also require form)
- ☐ Patient specimens (tissues, blood, serum, surgical discards, etc.)
- ☐ Other (specify):

C 2. For each of the boxes checked in 1, how were the original data, records, or human biological specimens collected? Describe the process of data collection including consent, if applicable.

C 3. For each of the boxes checked in 1, where do these data, records or human biological specimens currently reside?

C 4. For each of the boxes checked in 1, from whom do you have permission to use the data, records or human biological specimens? Include data use agreements, if required by the custodian of data that are not publicly available.

C 5. If the research involves human biological specimens, has the purpose for which they were collected been met before removal of any excess? For example, has the pathologist in charge or the clinical laboratory director certified that the original clinical purpose has been satisfied? Explain if necessary.

☐ yes ☐ no ☐ not applicable

C 6. Do all of these data records or specimens exist at the time of this application? If not, explain how prospective data collection will occur.

☐ yes ☐ no If no, explain